U.S. DEPARTMENT OF COMMERCE
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PB80-102338

Analysis of Airborne Viable Bacteria at Solid Waste Processing Facilities

Midwest Research Inst, Kansas City, MO

Prepared for

Municipal Environmental Research Lab -Cincinnati, OH

Aug 79

United States
Environmental Protection
Agency

Municipal Environmental Research
Laboratory
Cincinnati OH 45268

EPA-600/2-79-131
August 1979

Research and Development

PB80-102338

EPA

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TECHNICAL REPORT DATA
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1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/2-79-131		PB 80-102338

4. TITLE AND SUBTITLE	5. REPORT DATE
ANALYSIS OF AIRBORNE VIABLE BACTERIA AT SOLID WASTE PROCESSING FACILITIES	August 1979 (Issuing Date)
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
M. W. Fletcher and D. E. Fiscus	MRI Project No. 4033-L
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Midwest Research Institute 425 Volker Boulevard Kansas City, Missouri 64110	1NE624, SOS WF, Task 6.1
	11. CONTRACT/GRANT NO.
	68-02-1871
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Municipal Environmental Research Laboratory--Cin.,OH Office of Research and Development U.S. Environmental Protection Agency Cincinnati, Ohio 45268	Analytical Methods
	14. SPONSORING AGENCY CODE
	EPA/600/14

15. SUPPLEMENTARY NOTES
See also EPA-600/2-78-152 and EPA-600/2-79-090.
Project Officer: Carlton C. Wiles 513/684-7881.

16. ABSTRACT

This report presents a synopsis of aerobiological testing methodologies used by Midwest Research Institute during studies conducted for the Environmental Protection Agency. The purpose of the programs was to determine relative levels of airborne bacteria and virus aerosols within and around waste handling facilities. The facilities tested were the St. Louis Refuse Processing Plant, the Browning Ferris/Raytheon Service Company Resource Recovery Plant (Houston), and at three other types of waste handling facilities, i.e., an incinerator, a waste treatment plant, and a landfill. The report presents microbiological air sampling methodologies, field sample handling procedures, and laboratory analysis methodologies. It also includes a general discussion of pertinent considerations, recommendations for future research, and an extensive bibliography.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Bacteria Viruses Microorganisms Wastes Refuse disposal Air pollution	Laboratory methods Air emissions Ambient air Refuse derived fuel	13B

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
	Unclassified	92
Release to Public	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE PC A05 MF A01

EPA Form 2220-1 (Rev. 4-77)

☆ U.S. GOVERNMENT PRINTING OFFICE 1979 -657-060/5418

EPA-600/2-79-131
August 1979

ANALYSIS OF AIRBORNE VIABLE BACTERIA AT SOLID WASTE PROCESSING FACILITIES

by

M. W. Fletcher and D. E. Fiscus
Midwest Research Institute
Kansas City, Missouri 64110

Contract No. 68-02-1871

Project Officer

Carlton C. Wiles
Solid and Hazardous Waste Research Division
Municipal Environmental Research Laboratory
Cincinnati, Ohio 45268

MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is a necessary first step in problem solution, and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved systems technology to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research.

The St. Louis-Union Electric-Environmental Protection Agency refuse fuel project was the first use demonstration of solid waste as a supplementary fuel in power plant boilers for generating electricity. In addition to the fuel demonstration, research tasks were conducted to evaluate the relative levels of airborne bacteria and viruses at the St. Louis Refuse Processing Plant and other waste handling facilities for purposes of hazard assessment.

This report was prepared as an evaluation of the analytical methodologies used to determine airborne microbiological emissions. The total research program is fully discussed in the report, "Assessment of Bacteria and Virus Emissions at a Refuse Derived Fuel Plant and Other Waste Handling Facilities," EPA-600/2-78-152, U.S. Environmental Protection Agency, August 1978.

Francis T. Mayo, Director
Municipal Environmental Research
Laboratory

ABSTRACT

This report presents a synopsis of aerobiological testing methodologies used by Midwest Research Institute during studies conducted for the Environmental Protection Agency. The purpose of the programs was to determine relative levels of airborne bacteria and virus aerosols within and around waste handling facilities. The facilities tested were the St. Louis Refuse Processing Plant, the Browning Ferris/Raytheon Service Company Resource Recovery Plant (Houston), and three other types of waste handling facilities: an incinerator, a waste treatment plant, and a landfill. The report presents microbiological air sampling methodologies, sample handling procedures, and laboratory analysis methodologies. It also includes a general discussion of pertinent considerations with recommendations for future research, and an extensive bibliography.

This report is of interest to those involved in measuring airborne microorganisms at waste handling and processing facilities. A complete discussion of the entire research program is contained in the report entitled "Assessment of Bacteria and Virus Emissions at a Refuse Derived Fuel Plant and Other Waste Handling Facilities," EPA-600/2-78-152, U.S. Environmental Protection Agency, August 1978.

This report was submitted as part of Contract No. 68-02-1871, by Midwest Research Institute under the sponsorship of the U.S. Environmental Protection Agency and covers work done during the period 1975 through 1978.

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ACKNOWLEDGMENT

This report was prepared by Midwest Research Institute for the Municipal Environmental Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio, under EPA Contract No. 68-02-1871. The project officer for the Environmental Protection Agency was Carlton C. Wiles.

This report was written by Messrs. M. W. Fletcher and D. E. Fiscus. However, many of Midwest Research Institute's personnel contributed to the programs which resulted in this publication. Foremost among the contributors are Dr. F. Wells, Dr. W. Spangler, Mr. R. Flippin, Mr. P. Gorman, Mr. M. Golembiewski, and Dr. K. P. Ananth. Other contributors were Mr. B. DaRos, Mr. T. Merrifield, Mr. R. White, Mr. M. P. Schrag, and Dr. L. J. Shannon. The assistance and support of these scientists are gratefully acknowledged.

SECTION 1

SUMMARY OF RECOMMENDATIONS

Airborne microorganism emissions from refuse-derived fuel (RDF) and other waste handling facilities are not well characterized. Further research should be conducted to better define their qualitative and quantitative nature. The following summary of recommendations will aid future research attempts to characterize microbial aerosol emissions.

- Hi-Vol air samplers are not recommended to sample for airborne microorganisms because of the deleterious effects on microbial viability due to desiccation.

- AGI-30 impingers with gelatin-milk impinger fluid (or other suitable collection menstruum) are recommended for quantitative sampling for airborne bacteria and virus both in and around waste handling facilities.

- AGI-30 impingers should not be operated for periods greater than 10 to 30 min.

- Sampling for airborne microorganisms with an AGI-30 in the presence of large particulates may necessitate the addition of a dry modified Greenburg-Smith impinger preceding the AGI-30 impinger. The contents of both impingers should be analyzed if quantitative microbiological results are desired.

- The Andersen impactor is recommended only for microorganism counts by particle size. The Andersen impactor is not recommended for quantitative airborne microorganism concentrations because airborne particulates may contain more than one microbial cell. Multiple microbial cells per particle may result in only one colony count and the true count may thereby be underestimated.

- The Andersen impactor should not be operated for periods exceeding 30 min.

- AGI-30 impinger and Andersen impactor sampling devices should always be included as reference sampling devices if another sampler is to be principally used.

- Although not tested in these studies, the Litton large-volume air sampler (LVS) may be used to sample for specific airborne microorganisms, i.e., Salmonella, Staphylococcus, and Klebsiella, and viruses. These microorganisms may occur at threshold detection levels with the AGI-30 impinger, but still may be present at epidemiologically significant levels.

- Bacterial analysis of aerosol samples should be initiated within 4 hr of collection.

- Virus analysis of aerosol samples should be initiated as soon as possible. If delays are unavoidable, the samples should be quick-frozen and stored at -70°C.

- Only optimum media and/or methods which have undergone rigorous comparisons under identical conditions, preferably with aerosol samples, should be used to analyze aerosol samples for microorganisms.

- Monolayer plaque assay methods, with sensitive cell lines, are recommended for titration of viruses. HeLa or KB cell lines are recommended for titration of adenoviruses. Primary rhesus monkey kidney cells or the continuous monkey kidney cell lines BGM or LLC-MK_2 are recommended for enterovirus titration.

- Direct sample incorporation onto cell monolayers without prior sample concentration is recommended for virus assays of samples containing sufficiently large concentrations of viruses.

- The phase separation method of virus concentration is one technique recommended for samples containing low concentrations of viruses. Concentration efficiency controls should be conducted with a known virus strain.

- Use of microbial indicators of sanitary significance such as total coliforms, fecal coliforms, and fecal streptococci in determining relative levels of air hazard should be avoided. The significance of these indicators in the atmospheric environment has not been thoroughly established.

SECTION 2

INTRODUCTION

Concern that aerosol emissions might contain pathogenic microorganisms from industrial and public utility facilities has prompted new interest in aerobiological testing. Emissions assessments for airborne microorganisms have recently been conducted at sewage treatment facilities (1-8), food processing waste spray fields (9), cooling towers (10-12), and more recently solid waste handling facilities with associated refuse derived fuel plants (13,14).

Difficulties in quantitatively determining aerosolized microorganisms in controlled laboratory systems are well known. The problem of accurately assessing airborne microorganism concentrations under field conditions presents even greater difficulties due to the increased number of uncontrollable environmental variables. Additional quantitation variables arise from individual choices of aerosol sampling devices and laboratory analysis procedures.

Midwest Research Institute (MRI) recently conducted aerosol emission tests for bacteria and virus at the St. Louis-Union Electric Facility (14) (hereafter referred to as the St. Louis program) and at the Houston/Browning Ferris Industries (15) (hereafter referred to as the Houston program). Numerous problems were encountered during these programs, primarily concerning the nature of the particulate emissions at the refuse facilities tested and the microbial viability variables of the sampling methods employed.

The purpose of this report is to present the aerosol sampling and microbiological assay procedures used during the St. Louis and Houston programs. The report covers the air sampling devices, sample handling procedures in the field, and microbiological analysis procedures used during the programs. Problems encountered with the methods are discussed and recommendations are made with regard to future research. A bibliography is included to assist in future research efforts.

This report is primarily concerned with procedures and methodologies. Further information concerning the results obtained during the St. Louis and Houston programs may be found in the following reports:

- Assessment of Bacteria and Virus Emissions at a Refuse Derived Fuel Plant and Other Waste Handling Facilities (14).

- Evaluation of Fabric Filter Performance at Browning Ferris Industries/ Raytheon Service Company Resource Recovery Plant, Houston, Texas - Draft Report (15).

- Executive Summary - Assessment of Bacteria and Virus Emissions at a Refuse Derived Fuel Plant and Other Waste Handling Facilities (16).

- Dust and Airborne Bacteria at Solid Waste Processing Plants (17).

- Comparative Assessment of Bacterial and Viral Field Sampling Methods Used at Solid Waste Handling and Processing Facilities (18).

SECTION 3

AEROSOL SAMPLING DEVICES AND FIELD METHODOLOGY

ST. LOUIS PROGRAM

The major aerosol sampling device for airborne microorganisms during the St. Louis program was the Model GMWL-2000 Hi-Vol sampler.[a/] It was chosen as the primary sampling device because it could simultaneously sample aerosols for three parameters: microorganisms, physiochemical particulate morphology, and trace metals. Hi-Vol samplers placed at upwind and downwind locations were standard units. The Hi-Vol samplers used for in-plant sampling were standard units equipped with a Model 230 CP Cyclone Preseparator[b/] to remove large particulates and eliminate filter overloading, which has been reported as one of the Hi-Vol's major problems (19). All of the Hi-Vol samplers were operated at a continuous sampling rate of 1,134 liters/min for 6 hr with Gelman Type AE glass fiber filters[c/] serving as the collection medium.

Counts of airborne microorganisms by particle size were conducted with an Andersen Model 10-800 sampler[d/] coupled with a backup modified Greenburg-Smith impinger[e/] to remove any viruses passing the Andersen impactor. Plate count agar (27 ml) served as the collection medium for the Andersen impactor. The Greenburg-Smith impinger contained 100 ml Hanks' balanced salt solution (BSS) as the collection menstruum. The sampling train was operated at 27.96 liters/min for 0.5 min at in-plant sites and 10 min at property line sites.

Greenburg-Smith impingers were used alone during special sampling applications for airborne microorganisms. Again, the collection menstruum was 100 ml Hanks' BSS in all instances. The sampling rate was held constant at 27.96 liters/min for 6 hr at each sampling site.

---

a/ General Metal Works, Inc., 8368 Bridgetown Road, Cleaves, Ohio 45002.
b/ Sierra Instruments, P.O. Box 909, Carmel Valley, California 93924.
c/ Gelman Instrument Company, 600 South Wagner Road, Ann Arbor, Michigan 48106.
d/ Andersen Samplers, Inc., 4215-L Wendell Drive, Atlanta, Georgia 30336.
e/ Ace Glass, Inc., P.O. Box 688, Vineland, New Jersey 08360.

HOUSTON PROGRAM

The sampling devices were modified somewhat for the Houston program because of the experience gained during the St. Louis testing. Only AGI-30 impingers[f/] and the Andersen Model 10-800 sampler were used as sampling devices.

AGI-30 impingers were used to collect air samples for quantitative determinations of airborne bacteria and virus. At the baghouse inlet, the sampling train consisted of a 1.52 m (5.0 ft) stainless steel probe with a 2.54-cm ID (1.0-in.) probe tip, a dry modified Greenburg-Smith impinger, and an AGI-30 impinger containing 25.0 ml gelatin-milk solution (same as collection medium ii of White (20), but with omission of spermidine phosphate), a vacuum pump, and a dry gas meter. The Greenburg-Smith impinger served to trap large particles that might otherwise plug the AGI-30 orifice. For sampling at the baghouse outlet, the Greenburg-Smith impinger was omitted. Samples were taken for 30-min intervals at 4.8 liters/min (0.18 cfm) for the baghouse inlet and 3.6 liters/min (0.13 cfm) at the baghouse outlet. Samples at both sites were taken from single points in the duct.

Andersen microbial impactors were used to quantitate bacterial concentrations by particle size. Plate count agar (27 ml) containing 50 units Mycostatin[g/] antifungal agent per milliliter agar served as the collection medium. Samples were collected from a single point in the duct using the same probe as was used with the AGI-30 impingers. The baghouse inlet was sampled for 20 sec at a flow rate of 27.0 liters/min (0.951 cfm). Sampling times were increased to 10 min at the baghouse outlet to compensate for the lower particulate concentrations.

---

f/ Ace Glass, Inc., P.O. Box 688, Vineland, New Jersey 08360.

g/ Grand Island Biological Company, 3175 Staley Road, Grand Island, New York 14072.

SECTION 4

AEROSOL SAMPLE HANDLING PROCEDURES

The sample handling methodology was dependent upon the type of sampling equipment used. The following are procedure descriptions for each type of sampling device used.

ST. LOUIS PROGRAM

Hi-Vol Samplers

The Hi-Vol samplers were equipped with a filter support screen and an open metal frame with a foam gasket to seal the filter edges in place. The samplers were field-cleaned by wiping down the filter screen, metal frame, and surrounding area inside the Hi-Vol with lint-free chemical wipes saturated with 70% isopropyl alcohol. The residual alcohol was allowed to evaporate prior to installing the sterile filter. All manipulations of the sterile filter during installation and removal were made while wearing sterile vinyl gloves. After sampling, the filter was removed, placed in a sterile envelope, labeled, and stored in a refrigerator until sample shipment.

Hi-Vol Sampler with Precyclone

Sample handling procedures for the Hi-Vol with precyclone sampler were identical to the standard Hi-Vol with the exception of that used for the precyclone. The precyclone was cleaned of debris and cleaned with 70% isopropyl alcohol prior to installing a new filter and initiating sampling.

Andersen Impactor with Backup Impinger

Andersen impactors and Greenburg-Smith impingers were field-sterilized in a high-frequency ultraviolet light cabinet due to the unavailability of an autoclave and the necessity of equipment turnover. In addition, prior to ultravioloet irradiation, the impingers were supplementally cleaned with 70% isopropyl alcohol. After the technicians discarded the alcohol, the impingers were thoroughly rinsed twice with sterile Hanks' BSS prior to being filled with 100 ml fresh Hanks' BSS collection menstruum.

Lids to the Andersen agar plates were stored in the ultraviolet cabinet to prevent contamination during sampling. Following sampling, the Andersen plates were individually removed, covered with their lids, labeled, and taped together as a set. Impinger solutions were poured into labeled sterile-glass bottles and sealed. Samples were stored in a refrigerator until shipment.

Impingers

Procedures used for the special sampling Greenburg-Smith impingers were identical to those used for the Andersen backup impingers discussed in the preceding section.

Sample Shipment

Packaged samples were placed in insulated shipping cartons with plastic enclosed ice packages. Void spaces in the shipping containers were filled with packing to prevent sample damage during shipping. Shipping containers were strapped shut and sent by air express to MRI at the end of each sampling day.

HOUSTON PROGRAM

During the Houston program all materials were pre-sterilized at MRI and shipped by air to Houston. Sufficient equipment was available so that sampling devices were used only once in the field. The only piece of equipment reused in the field was the stainless-steel sampling probe. Prior to each use, the interior of the probe was thoroughly rinsed with absolute ethanol and carefully ignited for sterilization purposes. The ends were then covered with sterile squares of aluminum foil before transfer to the sample site. Individually bottled gelatin-milk impinger solution was aseptically added to the AGI-30 impingers with the aid of a propane torch on the day of sampling.

Upon completion of each sampling run, the volume of the AGI-30 was reconstituted with sterile distilled water and aseptically halved. One portion was transferred to a sterile sample bottle, quickly frozen, and shipped by air to MRI for viral analysis. The remaining portion was delivered in the impinger to MBA Laboratories in Houston so that bacterial analysis could begin promptly. Andersen impactor plates were packed in ice and sent to MRI on a daily basis.

During the initial tests at Houston, only the AGI-30 impinger solutions were analyzed at the baghouse inlet. This procedure produced erroneous quantitative results (15). During subsequent testing, two fractions at the baghouse inlet were collected and analyzed. The first fraction consisted of pooled sterile distilled water rinsings of: the probe tip to the Greenburg-Smith impinger; the connector tubing between the Greenburg-Smith impingers and AGI-30; and the Greenburg-Smith impinger interior. The second fraction consisted of the aseptically reconstituted volume of the AGI-30 impinger solution.

All impingers were kept in an ice bath during sampling, but the samplers were not shielded from direct sunlight during sampling.

SECTION 5

SAMPLE PREPARATION AND MICROBIOLOGICAL ANALYSIS

Microbiological samples obtained during the St. Louis program were analyzed exclusively at MRI's microbiolog labs. During the Houston program, however, only the bacteria count by si e (Andersen agar plates) and virus assays were completed at MRI's labs. Analysis of total bacteria and bacteria by species was conducted at MBA Laboratories, Houston, Texas. The analyses were split during the Houston program because it was desirable to start the bacterial assays within 4 to 6 hr of collection.

SAMPLE PREPARATION

St. Louis Program

The previous day's samples generally arrived in the lab shortly after 8:00 AM. The immediate sample processing procedure for the St. Louis samples is depicted in Figure 1. The Andersen agar plates were unpacked and placed inverted in a 35°C incubator. The Andersen backup impingers, one-half of the Air Density Separator (ADS) samples, and one-half of the Hi-Vol filters were unpacked and frozen at -70°C in a Revco Ultra-low Freezer for later viral analysis. The remaining halves of the ADS samples and Hi-Vol filters, along with the refuse samples and mobile filter impinger solutions, were processed immediately for bacterial analysis.

Fluid samples such as the mobile filter impingers were prepared by vigorous shaking and distributed in appropriate 1:100 dilutions. Solid samples such as the refuse samples, Hi-Vol filters, and ADS samples were diluted either 1:100 or 1:200 (w/w) with sterile distilled water and blended for 30 sec in a sterile Waring blender. The resulting homogenates were used directly or after subsequent 1:100 dilutions for inoculating the various media. The bacterial analysis spectrum is depicted in Figure 2.

Houston Program

During the Houston program, samples were shipped as soon as possible after collection either to MRI or to MBA Laboratories in Houston. The microbiology sample handling procedure is shown in Figure 3.

MICROBIOLOGY SAMPLE PROCESSING PROCEDURE
ST. LOUIS PROGRAM

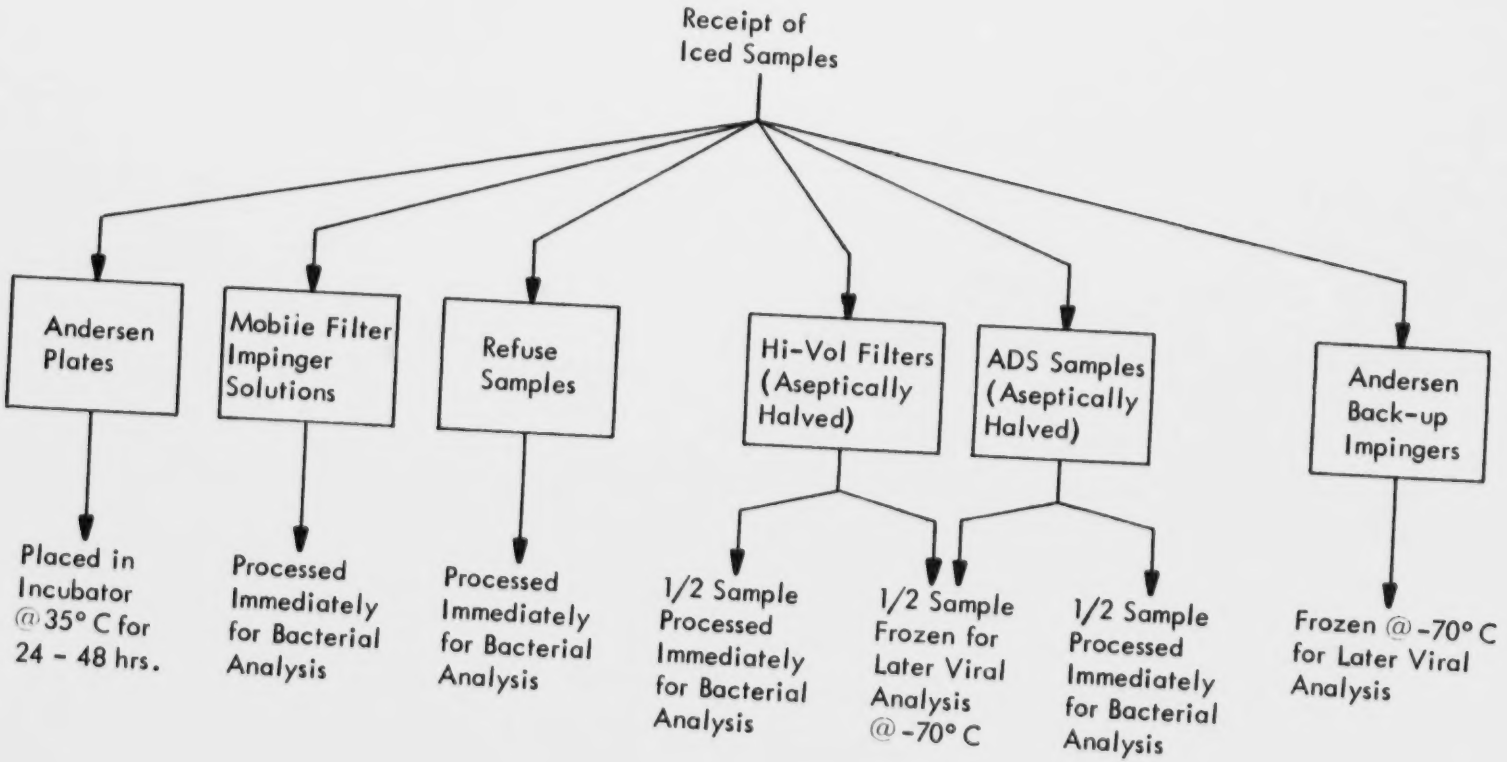
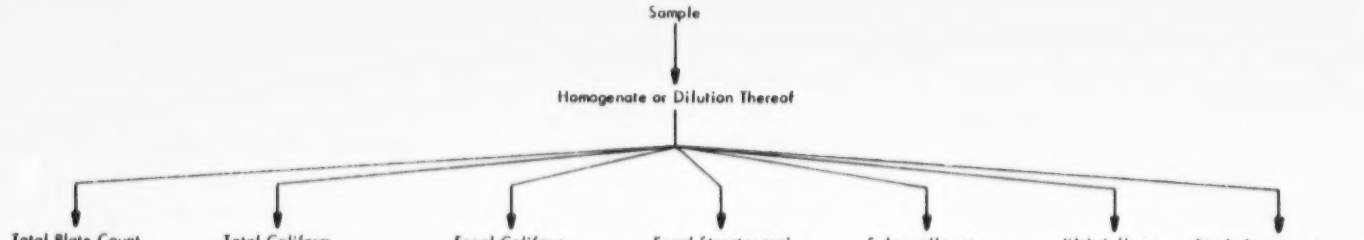

Receipt of Iced Samples
Andersen Plates
Mobile Filter Impinger Solutions
Refuse Samples
Hi-Vol Filters (Aseptically Halved)
ADS Samples (Aseptically Halved)
Andersen Back-up Impingers
Placed in Incubator @ 35° C for 24 - 48 hrs.
Processed Immediately for Bacterial Analysis
Processed Immediately for Bacterial Analysis
1/2 Sample Processed Immediately for Bacterial Analysis
1/2 Sample Frozen for Later Viral Analysis @ -70° C
1/2 Sample Processed Immediately for Bacterial Analysis
Frozen @ -70° C for Later Viral Analysis

Figure 1

BACTERIAL ANALYSIS SPECTRUM
ST. LOUIS PROGRAM



Method:	Pour Plate	MPN (5-10, 5-1, & 5-0.1 ml)	MPN	Pour Plate	Spread Plate & Enrichment	Spread Plate	Spread Plate
Media:	PCA	Lauryl Tryptose Broth & Brilliant Green Lactose Bile Broth	Lauryl Tryptose Broth & EC Broth	KF Streptococci Agar + TTC	MacConkey Agar & Brilliant Green Agar Selenite Broth	MacConkey Agar	Staphylococcus 110 Medium
Incub. Time:	48 hrs.	24 - 48 hrs.	24 hrs.	48 hrs.	24 - 48 hrs.	24 - 48 hrs.	24 - 48 hrs.
Incub. Temp:	35°C	35±0.5°C	44.5±0.2°C	35±0.5°C	35°C	35°C	37°C
Level 2 Tests: (Proposed)	None	None	Enteropathogenic Serotypes Isolation of Rep. Colonies on EMB - Nutrient Agar Slants *E. coli* OK Polyantiserum Slide Agglutination	None	Serotyping	Serotyping	Coagulase Production

Figure 2

MICROBIOLOGY SAMPLE HANDLING PROCEDURE
HOUSTON PROGRAM

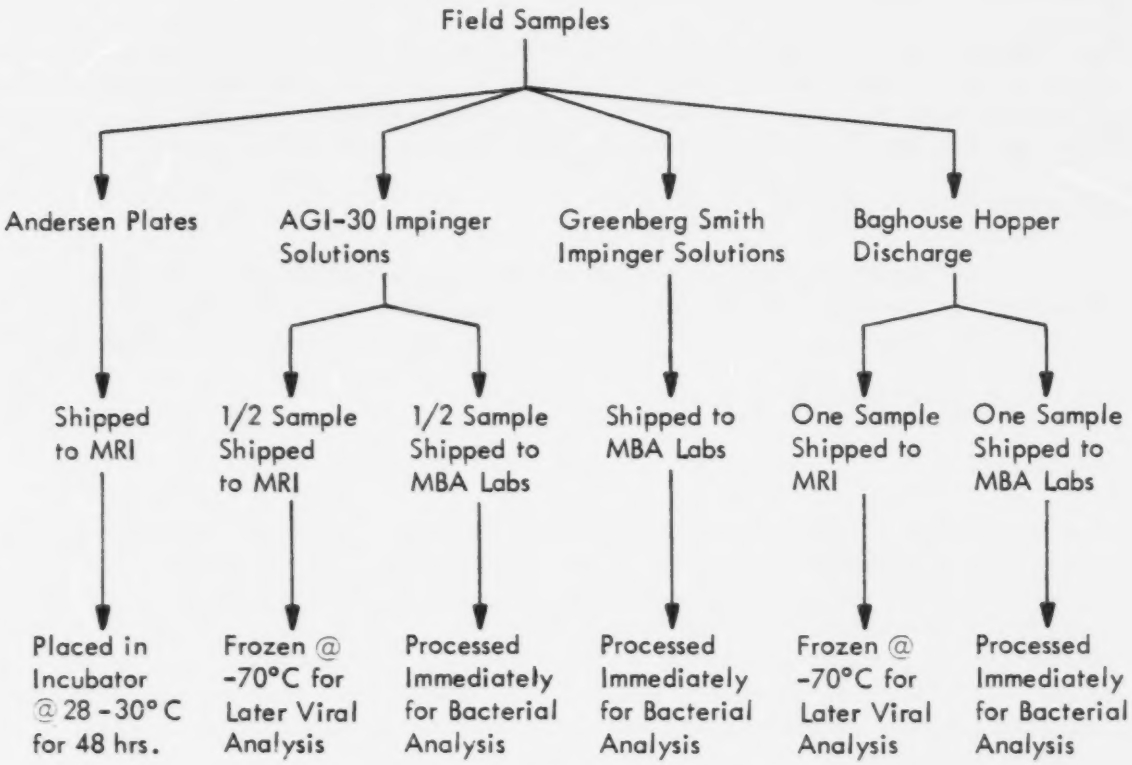

Field Samples
Andersen Plates
AGI-30 Impinger Solutions
Greenberg Smith Impinger Solutions
Baghouse Hopper Discharge
Shipped to MRI
1/2 Sample Shipped to MRI
1/2 Sample Shipped to MBA Labs
Shipped to MBA Labs
One Sample Shipped to MRI
One Sample Shipped to MBA Labs
Placed in Incubator @ 28 - 30° C for 48 hrs.
Frozen @ -70°C for Later Viral Analysis
Processed Immediately for Bacterial Analysis
Processed Immediately for Bacterial Analysis
Frozen @ -70°C for Later Viral Analysis
Processed Immediately for Bacterial Analysis

Figure 3

One-half of the AGI-30 impinger samples for virus analysis, Andersen impactor plates, and baghouse hopper discharge samples was shipped to MRI for analysis. The AGI-30 impinger and baghouse hopper discharge samples were stored frozen at -70°C until virus analysis could be initiated. Andersen plates were unpacked and placed inverted in a 28 to 30°C incubator.

The remaining halves of AGI-30 impinger, Greenburg-Smith impinger, and baghouse hopper discharge samples were hand-carried to MBA Laboratories within 2 to 3 hr of sampling. Bacterial analysis by MBA Laboratories was initiated immediately upon receipt of the samples.

Fluid samples such as the impinger solutions were used to inoculate media as received or after a 1:6 dilution. Solid samples such as the baghouse hopper discharge were diluted 1:90 (w/w) and vigorously shaken. The bacterial analysis spectrum for the Houston samples is depicted in Figure 4.

MICROBIOLOGICAL ANALYSIS PROCEDURES

Aerosol samples obtained during the St. Louis and Houston programs were analyzed for general and specific microorganism content (see Figures 2 and 4). The following section presents the analytical methods used during analysis.

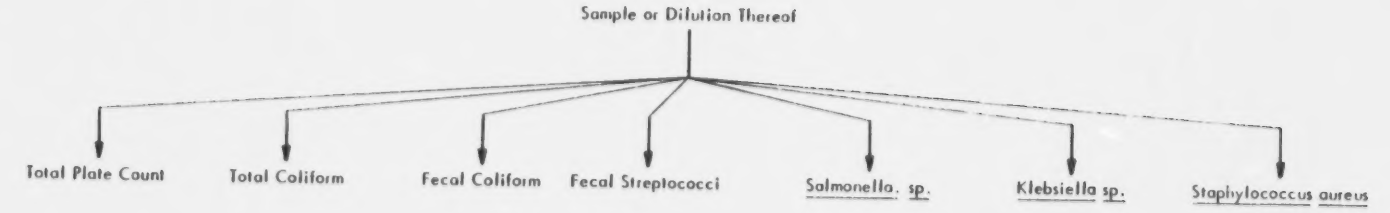
Bacteria Count by Size (Andersen Agar Plates)

St. Louis--

Plate count agar (Difco) in polystyrene petri plates (Falcon) served as the collection medium for the Andersen aerosol samples. The plates were poured to a depth of 27 ml with the aid of a sterile Cornwall syringe and allowed to solidify. After solidification, excess moisture on the agar surface was evaporated by placing the plates in a sanitized laminar flow unit for approximately 20 min with the lids partially removed. The plates were then repacked in their original packing sleeve and sealed with tape for shipment to the field.

Upon receipt of the inoculated Andersen agar plate samples at the lab, the plates were unpacked and incubated in an inverted position at 35°C for 24 to 48 hr depending upon the degree of mold contamination. After this period, bacterial counts were made on the basis of colonial morphology with the aid of a Quebec colony counter. Select representative colonies were picked at random from some of the Andersen plates and saved on typticase soy agar slants. These cultures were then used for determination of morphology using gram staining techniques.

BACTERIAL ANALYSIS SPECTRUM
HOUSTON PROGRAM



	Total Plate Count	Total Coliform	Fecal Coliform	Fecal Streptococci	Salmonella. sp.	Klebsiella sp.	Staphylococcus aureus
Method:	Spread Plate	MPN (3-10.0, 3-1.0, & 3-0.1 ml)	MPN	Spread Plate	Modified MPN	Enrichment MPN	MPN (3-1.0, 3-0.1, 3-0.001, & 3-0.0001 ml)
Media:	Plate Count Agar	Lactose Broth & Brilliant Green Lactose Broth	EC Broth	KF Streptococcal Agar + TTC	Lactose Broth Selenite Cystine Broth Salmonella-Shigella Agar Bismuth Sulfite Agar	Lactose Broth EMB Agar	Trypticase Soy Broth with 10% NaCl. Baird Parker Agar, Etc.
Incub. Time:	24 hrs.	24 - 48 hrs.	24 hrs.	48 hrs.	24 - 48 hrs.	24 - 48 hrs.	48 hrs.
Incub. Temp:	35°C	35°C	44.5°C	35°C	35°C	35°C	35°C

Figure 4

Houston--

The Andersen aerosol plate count methods used on the Houston program were the same as the St. Louis program with only a few modifications. In an effort to control molds from overgrowing and masking the bacterial count, the antifungal agent Mycostatin (nystatin) (21) was incorporated in the plate count agar at 50 units/ml agar. The agar plates were incubated at 28 to 30°C for 48 hr prior to determining the following microbial counts: total count, bacteria, yeast, mold, and antinomycetes. The categorical microbial counts were separated from the total count on the basis of colony morphological characteristics. It should be noted that the categorical counts can be used as a guide only to the relative population number. To obtain more absolute values, special sampling and assay considerations would have to be implemented for each microbial type. Isolates were not saved for morphology or gram-reaction determinations.

Total Plate Count

St. Louis--

Total plate counts were determined according to Standard Methods (22). Analyses of the filter pad slurry were determined in duplicate by the pour-plate method with plate count agar. The solidified plates were incubated aerobically at 35°C for 48 hr. Longer incubation times at a lower temperature might have given slightly higher counts but because mold spores were present in large numbers, it was not possible to extend the incubation time beyond 48 hr without overgrowth by fungi.

Isolated colonies were picked from representative plates and transferred to trypticase soy agar slants. The cultures were then used for determination of morphology and gram reaction.

Houston--

Total plate counts were conducted according to procedures detailed in Standard Methods (22). The impinger samples were not diluted. Solid samples were diluted 1:90 (w/w) and then homogenized. The samples were then analyzed by the spread-plate method, using plate count agar. Plates were incubated aerobically at 35°C for 24 hr prior to determining colony counts.

Standard Total Coliform MPN Tests

All coliform tests were conducted according to Standard Methods (22), using most-probable-number (MPN) techniques.

St. Louis--

Presumptive coliform tests during the St. Louis program were conducted using lauryl tryptose broth (LST) as the enrichment medium. Five fermentation tubes each of 10, 1, and 0.1 ml of the filter pad slurry were prepared and incubated at 35°C. At the end of 24 hr, each tube was examined, and those showing gas were recorded. Tubes showing no gas at 24 hr were reincubated for an additional 24 hr for a total of 48 hr. Formation of gas within 48 hr constituted a positive presumptive test.

Confirmation of total coliforms was conducted by subculturing to brilliant green lactose bile broth (BGLB) all LST tubes showing gas in 24 or 48 hr in the presumptive test. The BGLB tubes were then incubated at 35°C for 48 hr. Formation of gas within 48 hr constituted a positive confirmed test.

Total coliform completion tests were conducted by streaking aliquots of each BGLB tube showing gas onto eosin-methylene-blue (EMB) agar plates. The EMB plates were incubated at 35°C for 24 hr and then observed for typical colonies (nucleated with or without metallic sheen). Typical colonies were subcultured on nutrient agar slants and examined after 24 hr by use of the gram-stain technique. Gram-negative cultures were considered to be coliform.

Houston--

Total coliform counts during the Houston program were also determined by the MPN technique. Solid samples were diluted 1:90 (w/w) and the impinger samples 1:6. A lactose broth three-tube/three-dilution series of 10, 1.0, and 0.1 ml was used for both liquid and solid samples. After incubation at 35°C for 48 hr, all tubes showing gas were transferred to BGLB for confirmation of positive reaction. Coliform tests were not completed.

Fecal Coliform MPN Tests

St. Louis--

Fecal coliform tests (22) during the St. Louis program were conducted on all LST tubes showing gas in the presumptive coliform test. The LST tubes were used to inoculate tubes of EC medium. The EC tubes were then incubated in a water bath at 44.5°C for 24 hr. Gas production in 24 hr constituted a positive test and indicated the presence of coliform microorganisms of fecal origin. Most probable number tables were used to determine probable fecal coliform densities in the original sample.

Subcultures were made from positive EC tubes to EMB agar, and typical colonies on the solid medium were then transferred to nutrient agar slants and saved for serological typing for enteropathogenic Escherichia coli.

Houston--

Fecal coliform counts were determined by the MPN technique (22). All lactose tubes from the total coliform tests which showed gas production (positive reaction) were used for fecal coliform analysis. EC media were inoculated from each positive lactose tube and incubated at 44.5°C for 24 hr. Gas production within 24 hr represented a positive test. Coliform densities were determined from standard MPN tables.

Serological Typing of *Escherichia coli*

St. Louis--

Subcultures from nutrient agar slants were made to brain-heart infusion agar (BHIA) of typical fecal coliform (EC) isolates. The BHIA cultures were incubated overnight, and on the following day portions of each culture were serologically tested against *E. coli* OK antiserum (poly) by the slide agglutination technique as described by Difco Laboratories (23).

Fecal Streptococci

St. Louis--

Fecal streptococci were enumerated by a pour-plate technique utilizing KF streptococci agar containing 0.01% 2,3,5-triphenyltetrazolium chloride (TTC). Colonies appearing dark red or pink were counted as fecal streptococci.

Houston--

Fecal streptococci were enumerated by the spread-plate technique used with KF streptococcal agar and TTC. Plates were incubated at 35°C for 48 hr. All suspected colonies were examined microscopically for confirming morphology.

Salmonella

St. Louis--

Salmonella tests were conducted on filter-pad slurry by direct-spread plate and enrichment broth-spread plate techniques. Slurry-sample and 24 to 48 hr selenite broth enrichment aliquots were transferred to both MacConkey and brilliant green agars. Typical colonies appearing colorless on MacConkey agar and red or pink to nearly white on brilliant green agar were transferred to triple sugar iron (TSI) agar slants. Cultures giving typical reactions for *Salmonella* on TSI agar were transferred to trypticase soy agar slants and retained for serological testing.

Salmonella isolates were serologically tested using the slide agglutination technique (23) with salmonella polyvalent antisera. Positive reactors were then tested using salmonella group specific antisera.

Houston--

One-milliliter lactose broth aliquots from the coliform analysis procedure were inoculated into selenite cystine broth (SCB) medium. After 24 hr incubation, inocula from SCB tubes were streaked onto salmonella-shigella (SS) agar and incubated at 35°C for 48 hr. Typical salmonella colonies on SS agar were picked and transferred to TSI slants. Following incubation, slants showing typical salmonella reactions were tested for confirming biochemical reactions in the following media: lysine decarboxylase, dulcitol, sucrose, lactose, malonate, citrate, indole, and motility.

Klebsiella

St. Louis--

Tests for Klebsiella were conducted on filter pad slurry dilutions. The spread-plate technique was used on MacConkey's agar. Typical red, mucoid-appearing colonies were picked and transferred to additional MacConkey and EMB agar plates for purity. Typical appearing colonies were transferred to BHIA to serve as stocks to determine their respective biochemical reactions using the API 20E system.[h/] Isolates possessing biochemical characteristics similar to those reported for Klebsiella sp. were serologically tested with polyvalent klebsiella antiserum.

Houston--

Positive lactose tubes from the coliform analysis procedure were streaked onto EMB agar plates and incubated at 35°C for 24 hr. All colonies morphologically identified as typical Klebsiella were inoculated onto TSI slants. The TSI slants exhibiting an acid slant with an acid butt, with or without gas, were further tested for ornithine decarboxylase, indole, motility, and urease. Only those isolates exhibiting typical klebsiella reactions were reported as Klebsiella sp.

Staphylococcus aureus

St. Louis--

The presence of Staphylococcus aureus was determined by spread-plate technique on staphylococcus 110 agar plates. These plates were incubated at 37°C for 24 and 48 hr; all typical appearing colonies (light yellow to orange

h/ Analytical Products, 200 Express Street, Plainview, New York 11803.

pigment) were transferred to brain-heart infusion slants. After overnight growth, the isolates were tested for production of coagulase by the rabbit serum EDTA coagulase tube test.

Houston--

S. aureus was determined by the MPN procedure detailed in the A.O.A.C. Manual (24) A four-dilution/three-tube series of trypticase soy broth with 10% sodium chloride was inoculated and incubated at 35°C for 48 hr. Tubes showing growth were streaked onto Baird Parker agar plates and incubated for another 48 hr at 35°C. Plates were then examined for suspect colonies which were transferred to brain-heart infusion broth (BHIB). The BHIB tubes were incubated at 35°C for 24 hr, after which aliquots were subjected to the rabbit serum EDTA coagulase tube test. Tubes showing a positive reaction after 4 hr were reported as S. aureus.

Virus Methodology

St. Louis Program--

Samples for virus analysis were concentrated prior to virus titration. Initially, samples were concentrated by the hydroxyapatite method. This concentration method was replaced early in the research by the more efficient dextran sulfate-polyethylene glycol phase separation method of Shuvall (25) (see Table 1). Most of the samples were concentrated by the phase separation method.

Concentrated samples were analyzed for virus content by the monolayer plaque assay technique of Schmidt (26) with minor modifications (see Table 2). In all samples, 5- to 7-day old confluent 75 cm^2 monolayers of heteroploid monkey kidney cells, LLC-MK_2 (GIBCO) were used. The monolayers were grown at 35°C in Earles' M-199 containing glutamine and supplemented with 10% fetal calf serum, 0.22% $NaHCO_3$ for stoppered incubation, GIBCO's antibiotic-antimycotic amendment (penicillin, streptomycin, and fungizone) and GIBCO's anti-PPLO amendment (tylocine).

Because of severe mold contaminations, samples for virus titration were diluted 1:10 in Earles' BSS. One milliliter of the 1:10 sample dilution was distributed over the monolayer after pouring off the growth medium. The flask was incubated for 30 min at 37°C to allow virus attachment. The inoculum was poured off and, in a darkened room, replaced with 10.0 ml of molten overlay medium at 45 to 47°C consisting of the growth medium supplemented with 1.7 x 10^{-3}% neutral red and 1.5% ion agar No. 2. The overlay was quickly distributed over the monolayer and placed on a flat surface for 30 min to allow solidification of the agar. The flasks were firmly sealed and incubated in the dark at 36 to 37°C. The tissue cultures were checked for plaque formation at 4, 7, and

TABLE 1. FLOW SHEET FOR VIRAL CONCENTRATION PROCEDURES OF AEROSOL SAMPLES ON FILTER MEDIA[a/] (Phase Separation Method)

Homogenize weighed sample after diluting 1:100 (w/w) with distilled water.

↓

Centrifuge at 1,080 x g for 15 min at 4°C.

↓ → Discard Ppt.

Neutralize supernatant to pH 7.2.

↓

Add each of the following sequentially after each is thoroughly dissolved:

1.75% (w/w) (0.3 M) Dry sodium chloride
0.2% (w/w) Sodium dextran sulfate 2000
6.43% (w/w) Polyethylene glycol 4000

↓

Allow to mix 1 hr using a magnetic stirrer.

↓

Transfer mixture to separatory funnel and store at 4°C for 18 to 24 hr.

↓

Collect bottom and interphase portions.

↓

Follow the method below: (after Shuvall, H. I. et al., 1969)

To bottom and interphase portions, add KCl to 5.22% (w/w) (0.7 M) to ppt. dextran sulfate.
Centrifuge at 2,500 g for 10 min at 4°C.
To supernatant, add 1.0 ml anesthetic grade diethyl ether per 4 ml reconcentrate.
Shake mixture and hold at 4°C for 18 hr to kill contaminating bacteria and molds.
Tissue culture assay for viruses.

a/ This procedure may be modified to process impinger samples by eliminating the initial homogenization and centrifugation steps.

TABLE 2. MONOLAYER PLAQUE ASSAY TECHNIQUE[a/]

1. Remove growth medium from 5- to 7-day old monolayers in 75 cm^2 surface area flasks and rinse with three 8 ml portions of Earles' balanced salt solution.

2. Check monolayer for confluency.

3. Pipet 1.0 x 10^{-1} ml dilution of sample onto monolayer. Distribute the inoculum over the monolayer and incubate for 30 min at 37°C.

4. Pour off inoculum.

5. Pipet and evenly distribute 10.0 ml of the following nutrient overlay solution held at 45 to 47°C.

	ml	Final conc.
10X Earles' M-199 + Glutamine (GIBCO)	10.0	1X
Fetal Calf Serum (inactivated at 56°C for 30 min)	10.0	10%
7.5% $NaHCO_3$	3.0	0.22%
Neutral Red (1:300)	0.5	1.7 x 10^{-3}%
10X Antibiotic-Antimycotic (penicillin, streptomycin, fungizone, GIBCO)	1.0	1X
10X Anti-PPLO (tylocine, GIBCO)	1.0	1X
2% Ionagar No. 2 in double distilled water	74.5	1.5%

6. Place bottles on a flat surface in a darkened room for 30 min to permit the agar to solidify then firmly seal flasks.

7. Incubate the flasks in the dark at 36 to 37°C.

8. Check flasks for plaque formation at 4, 7, and 10 days.

a/ After: Reference 26.

10 days. Suspected plaques were picked and transferred to fresh monolayers for determining plaque reproducibility. Only reproducible plaques were considered to be of viral origin.

Houston Program--

Samples for virus analysis during the Houston program were handled similar to those during the St. Louis program. Only differences will be stressed here.

Houston samples were concentrated exclusively by the phase separation method of Shuvall (25). The concentrated samples were analyzed by the monolayer plaque assay technique with confluent 75 cm^2 monolayers of three cell lines: 7-day cultures of heteroploid monkey kidney cells (LLC-MK_2), 3-day cultures of a neoplastic nasopharnyx cell line (KB), 3-day cultures of a primary human fetal lung fibroblast line (similar to WI-38). Both positive and negative controls were run with field samples using the same concentration and analysis procedure. Negative controls consisted of Earles' BSS and a sterile impinger blank. Positive controls were prepared by seeding an Earles' BSS blank and a sterile impinger blank with attentuated polio type 1 virus.

SECTION 6

DISCUSSION

GENERAL CONSIDERATIONS

The accurate assessment of microbiological air quality in ambient and confined air has proven difficult in the past. Part of the assessment problem is due to the diversity and variance in the number of: (a) microbiological forms found in air, (b) particulate matter of unknown size and composition, and (c) chemical composition of the air. In addition, evaluation of test data is further complicated by the diversity of sampling methods and analytical procedures employed by investigators in a variety of applications.

An accurate assessment of airborne microorganisms depends upon method sensitivity and reproducibility; these important quantitative considerations are influenced by many factors, including:

- The air sampling device employed;
- Ability to obtain large statistically valid sample volumes (this may necessitate large air volumes to be sampled by one sampler or an increase in the number of sampling devices at a given point);
- Maintenance of viable conditions for vegetative microorganisms during and following sampling;
- Disruption of the microbial particulate and microbial microcolonies so that an accurate estimation of the absolute population can be made;
- A minimum of sample storage time prior to assay;
- Proper choice of general, differential, or selective growth media for the microorganism of concern; and
- Proper choice of environmental growth variables for the organism of concern.

AEROSOL SAMPLING DEVICES

Currently, a variety of sampling devices are being used to sample for airborne microorganisms. Chief among those used today are: all glass impingers (AGI-30), Andersen multistage impactors, cascade impactors, and Litton Large Volume Samplers (LVS). These devices and other sampling considerations are described elsewhere in more detail (27-33).

Biological collection efficiencies of airborne microorganisms sampling devices vary greatly because of complex interactions of many variables. Some of these variables are:

- Physiological stage of the microorganisms, i.e., vegetative cells or spores;
- Physiological and structural state of the microorganisms, i.e., normal or sublethally stress-damaged cells;
- Method of collection by the sampling device, i.e., impaction, impingement, filtration, etc.;
- Volume of air sampled; and
- Environmental conditions during sampling.

Biological efficiencies may be altered because of the sampling device's effect on microbial viability both during and following sampling. Complex and often deleterious stressing effects such as desiccation, rehydration, oxidation, radiation, osmotic shock, impaction shock, length of sampling time, collection menstruum, sample retention time, etc., contribute to decreases in biological efficiencies during aerosol sampling. Also, collection efficiencies and stressing effects vary both between and within diverse microbiological groups that may be assayed, i.e., bacteria, yeasts, molds, actinomycetes, or viruses which require different considerations altogether.

Standard reference samplers for microbial aerosols have been recommended. General agreement was reached at the First International Symposium on Aerobiology (34) that the AGI-30 and the Andersen samplers be used as standard reference samplers. In both cases, it was emphasized that methodology, i.e., techniques and materials, be rigorously and completely specified when reporting the results. The use of reference sampling devices with complete specification of methodology has been a problem in the past. Unfortunately, the problem remains today. Defense of altered methodologies may rest on the undebatable premise that environmental variables, type of information sought, or research cost often necessitate individual alteration of published methodologies. However, it must be stressed that if altered methodologies are used by different

investigators, this can and often does prevent meaningful comparisons of results even if the same type of sampling device is used.

During the St. Louis program, the Hi-Vol air sampler was chosen as the primary aerosol sampling device for several reasons. First, the Hi-Vol sampler could sample large volumes of air (1,000 to 1,200 liters/min), which theoretically would increase the representativeness of the samples obtained without increasing the number of samples taken at a single location. Second, the samples obtained by the Hi-Vol sampler would be used simultaneously for three separate parameters to be measured, i.e., microorganisms, physiochemical particulate morphology, and trace metals. Both of these features made the Hi-Vol sampler attractive in terms of the time and manpower savings. Unfortunately, the Hi-Vol air sampler was run for 6 hr as a compromise for the non-microbiological parameters to be assessed. As a result, the microbiological samples suffered severe exposure to desiccation and oxidation factors. Unfortunately, AGI-30 impingers were not run concurrently as references at St. Louis. The experience gained, however, was applied on the Houston program.

The AGI-30 impinger and Andersen impactor should always be used as reference airborne microorganism sampling devices during aerosol emission research (34). Quantitative airborne bacteria and virus concentrations should be assessed with the AGI-30 impinger containing a collection menstruum compatible with the microorganism(s) sought. Gelatin-milk or one-half strength tryptic soy broth should be used as the collection menstruum for bacteria. Gelatin-milk or tissue culture medium with serum should be used for viruses. In all instances, menstruum compatibility tests should be conducted with a suitable test microorganism prior to use in order to determine effects on microbial viability.

Sampling times for microbial aerosols should be closely monitored. Sampling times with the AGI-30 impinger should be for 10 min or less. Absolute maximum sampling times with an AGI-30 should be 30 min. Prolonged sampling may cause significant microbial die-off as a result of complex sampling effects. Regardless of sampling time, it is advisable to set up control sampling devices inoculated with an appropriate test microorganism to ascertain the effects of sampling under field test conditions. In sampling areas containing large particulates, it may be necessary to add a dry Greenburg-Smith impinger prior to the AGI-30 (as was done at Houston) to prevent clogging of the AGI-30 orifices. Rinsings of both impingers and their connector tubing should be included for quantitative analysis.

The Andersen sampler is recommended only for sizing aerosol particles containing microorganisms. The Andersen sampler should not be used for quantitative microbial aerosol concentrations because a colony resulting from one aerosol particle may have contained more than one microbial cell. Sampling times with the Andersen impactor should not exceed 30 min. Prolonged sampling at waste handling facilities may result in excessive build-up of sample on the

sieve plates thereby altering particle sizing efficiency. In addition, prolonged sampling may cause desiccation of the microbiological medium employed leading to altered concentrations of the medium's components. Increased concentration of media components over the recommended level may lead to toxic levels especially if selective media are used. As with the AGI-30, it is recommended that control sampling devices be set up with appropriate test microorganisms to determine the effects on microbial viability of sampling under field test conditions.

The Andersen sampler might also be considered as a quantitative sampling device for airborne viruses. Andersen samplers have been modified and used as samplers for airborne viruses (39-42).

It is recommended that both Andersen and AGI-30 sampling devices be shielded from direct exposure to sunlight. Research by Fedorak (35) has shown that significant microbial losses are encountered from exposure of sampling devices to direct sunlight during sampling.

Although not specifically tested during the St. Louis or Houston programs, results indicate that the Litton LVS may be included especially in sampling for specific low concentration microorganisms such as Salmonella sp., Klebsiella sp., Staphylococcus aureus, and viruses. The LVS Model M is capable of sampling at 1,000 liters/min, which is 35 times greater than the Andersen sampler (28.3 liters/min) and 80 times greater than the AGI-30 (12.5 liters/min). The significance of using the LVS to detect viruses at epidemiologically significant low concentration has been demonstrated (36-38). The LVS is currently marketed by Sci-Med Environmental Systems, Inc.[i/]

It is urged that extreme diligence be shown in reporting all aspects of the sampling procedure. It is also important to specify what the final sample constitutes, i.e., the collection medium only, the collection medium plus rinsings of the connector tubing (if applicable), etc.

MICROBIOLOGICAL ANALYSIS OF AEROSOL SAMPLES

Microbiological analysis of aerosol samples should be initiated as soon as possible after collection. It is desirable that sample analysis begin within the upper limit of 4 to 6 hr to avoid alteration of microorganism concentration due to growth (increase in number) or die-off. Sample analysis delays were encountered during the St. Louis program because of logistical problems. During the Houston program, these problems were alleviated by contracting a qualified local laboratory to conduct the assays. The alternatives would be to maintain a field laboratory or rent space at a local hospital or school.

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i/ 2411 Pilot Knob Road, St. Paul, Minnesota 55120.

If delays are unavoidable, bacteriological samples can be stored in ice up to 4 hr; however, die-off needs to be considered. Samples for virus analysis should be quickly frozen in dry ice or liquid nitrogen and stored at -70°C until analysis.

Standardized analytical procedures have been established for the microbiological analysis of food (24,43,44); water (22); solid waste (45); and clinical specimens (46-50). However, standard analytical procedures for microbial aerosol samples have not been established. The unique stresses to which aerosolized microorganisms are exposed present difficulties in choosing an optimal medium for their recovery. General growth media would provide, perhaps, a greater recovery of aerosol-stressed microorganisms than would differential or selective media. However, the advantages of general media are often lost by overgrowth of the desired vegetative microbial forms by the more resistant and perhaps less important spore-formers.

BACTERIOLOGICAL ANALYSIS

Numerous differential and selective media have been developed for assessment of genera or classes of microorganisms. Yet, even with optimal samples, qualitative accuracy and quantitative recovery will vary with media type. Comparisons of media for recovery of specific microorganisms are relatively common in the literature for clinical, food, and water samples, etc. Although these comparisons may be used as guidelines for media selections for assay of microbial aerosol samples, they cannot be applied with certainty because of the unique stresses exerted on aerosolized microorganisms. The comparison of media for the enumeration of Staphylococcus aureus in aerosol samples by Smith (51) is one of the few such studies applied to aerosol samples. Clearly this is an area of aerobiological research that needs further amplification.

Indicator microorganisms such as total coliforms, fecal coliforms, and fecal streptococci have been used for a number of years for assessing microbial quality of food and water. Indicators are particularly cost-effective where the pathogenic species, whose presence are used to indicate, are present at low levels and the cost of sensitive methods are prohibitive. In the absence of microbial air standards or thorough research methods needed to establish them, indicator species may be of value in determining relative levels of air hazard subject to further research.

Microbiological indicator systems such as total coliforms, fecal coliforms, and fecal streptococci are currently being used to assess ambient and confined air quality levels (13,14). Since these indicators possess real problems in assessing quality of the aqueous systems for which they were developed, use of such indicators in air quality assessments is open to conjecture. Coliform organisms have been found associated with a wide variety of nonfecal environmental sources including vegetation (53), soils (54), and insects (53,55).

Aerosols from these common sources may be generated easily and may cause erroneously high background counts. In addition, solid waste retention times may alter the original ratio of coliforms to pathogens. Further research is essential to clarify the meaning of conventional microbial indicators in the atmospheric environment and to establish their relationship to pathogens in that medium, if the indicators are to be of value in assessing microbial air hazard.

Research should be conducted to ascertain optimal media-methodology combinations for quantitation of aerosol-stressed microorganisms. During the St. Louis and Houston programs, reliance was placed on standard microbiological procedures. Until research is conducted comparing the various media of choice, under identical conditions with field aerosol samples, it is recommended that several types of media/methods be used concurrently. If this is not possible, emphasis should be placed on media/methods which have undergone rigorous comparisons under identical conditions for optimal differential, selective, and quantitative abilities. The research programs of Gabis (56), Raymond (57), and Niskanen (58) are good examples of studies of this nature, even though the studies are not concerned with aerosol samples.

At the present time, MRI recommends the bacteriological methods outlined in Table 3 for analysis of aerosol emission samples obtained at waste handling facilities. It should be noted that some of the procedures represent modifications of the ones used during the St. Louis and Houston programs.

VIRUS ANALYSIS

Previous research during the earlier 1975 St. Louis test program (unpublished) established that virus concentration procedures were necessary for virus-containing aerosol samples obtained at solid waste-type facilities. Initial samples for virus analysis during the St. Louis program were concentrated according to the hydroxyapatite method as conducted during the 1975 program. Concern over concentration efficiency of the hydroxyapatite method led to comparisons between the hydroxyapatite and dextran sulfate-polyethylene glycol phase separation method (25) with both T-1 bacteriophage and attenuated polio virus type 1. With bacteriophage T-4, percent recovery of the phase separation method was 18.2% as compared to 0.1% for the hydroxyapatite method. The phase separation method gave 23.9% recovery with polio virus type 1 as compared to 0.1% for the hydroxyapatite method. These tests proved the phase separation method to be significantly more efficient. As a result, the phase separation method was used to concentrate the remainder of the samples.

A large number of virus concentration procedures have been described in the literature. These concentration procedures can be divided into seven main groups (59): sample incorporation, ultrafiltration, freezing, two-phase separation, ultracentrifugation, electrophoresis, and adsorption and elution. The phase separation method was chosen over the other methods for several

TABLE 3. RECOMMENDED BACTERIAL ASSAY METHODS FOR RDF FACILITY AEROSOL SAMPLES

Assay	Method	Media	Incubation temp. (°C)	Incubation time (hr)	Verifications: Recommendation	Verifications: According to	Method reference
Total plate count or (count by particle size)	Pour or spread plate; Andersen plates	Tryptic soy agar + 50 units/ml Mycostatin (Nystatin)	28 - 35	48	-	-	Unpublished data and Parkinson (21)
Total coliform	MPN	Lauryl tryptose broth + brilliant green bile 2% broth	35	48	Recommended	Standard Methods (22)	Standard Methods (22)
Fecal coliforms	MPN	Lauryl tryptose broth + EC medium	35 44.5 ± 0.2	48 24	-	-	Standard Methods (22)
Fecal streptococci	Pour plate	KF streptococcus agar + 0.01% TTC	35	48	Recommended	Standard Methods (22)	Standard Methods (22)
Klebsiella Sp.	Spread plate	Levine EMB agar	35	24	Recommended	Ewing (50)	Ewing (50)
Salmonella Sp.	Enrichment	Lactose broth + selenite cystine broth + Bismuth sulfite agar	35 43 35	24 24 48	Recommended	Ewing (50) Gabis (56) Difco (23)	Gabis (56)
	Spread plate	Bismuth sulfite agar	35	48	-	-	-
Staphylococcus aureus	Spread plate	Baird Parker agar	35 - 37	48	Recommended	Rayman (57)	Rayman (57) Niskanen (58)

reasons. The method is recommended for concentrating samples containing viruses associated with particulates (22) as would be expected with aerosols. The method was the most promising to use with samples collected on filter media. Large or small volumes of sample can be concentrated by means of the phase separation method. The procedure is not difficult and time-consuming, and is thus conducive to processing large numbers of samples.

Many problems exist in the concentration of viruses at low levels from samples. To eliminate these problems, it is recommended that direct incorporation incorporation be the method of choice. If concentration of samples is unavoidable because of low virus content, the phase separation method is recommended for concentration of virus-containing samples.

Contamination of tissue culture monolayers by molds contained in the samples was a problem during the St. Louis and Houston programs. If severe mold or other microbiological contamination problems exist, they may be controlled to a limited extent by higher concentrations of antibiotics providing cell toxicity does not occur. Controls should be run to ensure that the antibiotic levels do not interfere with cell line viability and sensitivity.

Monolayer plaque assay methods according to Schmidt (26) are recommended for virus analysis of aerosol samples. Particular attention should be focused on virus sensitivity in the choice of cell lines for virus titration. For adenovirus titrations, HeLa or KB cell lines are recommended (15,60). Monkey kidney cell lines, e.g., BGM or LLC-MK_2, are recommended for titration of enteroviruses (14,61).

FUTURE WORK

Currently, ASTM Committee E-38.07 (62) is developing microbial air sampling and analysis standards for resource recovery plants. Development of standards for particular applications may well be the correct approach. However, general standards, included in all research studies at least as a reference so that studies may be validly compared, need also to be established.

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